Biomarkers Guidelines

- Based on the studies performed in CFS/ME to date and taking into consideration not to overlap other CDE subgroup topics, the CDE Biomarkers subgroup reviewed five categories of biomarkers:
    1. Microbiome/Microorganisms
    2. Proteome/Proteins
    3. Metabolome/Metabolism
    4. Genome/Epigenome
    5. Gene expression/Transcriptome

    All potential CFS/ME biomarkers identified to date under these five biomarker categories, per available literature, should be classified as EXPLORATORY.

- As the only publication studying minimum data elements for research reports on CFS (Jason et al., 2012)

    Jason LA, Unger ER, Dimitrakoff JD, Fagin AP, Houghton M, Cook DB, Marshall GD Jr, Klimas N, Snell C. Minimum data elements for research reports on CFS. Brain Behav Immun. 2012;26(3):401–406.

    Focuses on demographic and diagnostic elements, recommendations from alternative initiatives aimed at harmonizing data reporting and facilitating study replication in biomedical and biomarker research are adopted.

    BIOMARKER GENERAL CDE RECOMMENDATIONS
    McShane LM. In Pursuit of Greater Reproducibility and Credibility of Early Clinical Biomarker Research. Clin Transl Sci. 2017;10(2):58–60.

    It is important to collect information on dosage of medication(s) used prior to biomarker sampling.

    EQUATOR Network (Enhancing the QUAlity and Transparency Of health Research; http://www.equator-network.org/
    Simera I, Altman DG, Moher D, Schulz KF, Hoey J. Guidelines for reporting health research: the EQUATOR network's survey of guideline authors. PLoS Med. 2008;5(6):e139.

    MIBBI project (Minimum Information for Biological and Biomedical Investigations; http://mibbi.org)

Kettner C, Field D, Sansone SA, Taylor C, Aerts J, Binns N, Blake A, Britten CM, de Marco A, Fostel J, Gaudet P, González-Beltrán A, Hardy N, Hellemans J, Hermjakob H, Juty N, Leebens-Mack J, Maguire E, Neumann S, Orchard S, Parkinson H, Piel W, Ranganathan S, Rocca-Serra P, Santarsiero A, Shotton D, Sterk P, Untergasser A, Whetzel PL. Meeting Report from the Second "Minimum Information for Biological and Biomedical Investigations" (MIBBI) workshop. Stand Genomic Sci. 2010;3(3):259–266.

Taylor CF, Field D, Sansone SA, Aerts J, Apweiler R, Ashburner M, Ball CA, Binz PA, Bogue M, Booth T, Brazma A, Brinkman RR, Michael Clark A, Deutsch EW, Fiehn O, Fostel J, Ghazal P, Gibson F, Gray T, Grimes G, Hancock JM, Hardy NW, Hermjakob H, Julian RK Jr, Kane M, Kettner C, Kinsinger C, Kolker E, Kuiper M, Le Novère N, Leebens-Mack J, Lewis SE, Lord P, Mallon AM, Marthandan N, Masuya H, McNally R, Mehrle A, Morrison N, Orchard S, Quackenbush J, Reecy JM, Robertson DG, Rocca-Serra P, Rodriguez H, Rosenfelder H, Santoyo-Lopez J, Scheuermann RH, Schober D, Smith B, Snape J, Stoeckert CJ Jr, Tipton K, Sterk P, Untergasser A, Vandesompele J, Wiemann S. Promoting coherent minimum reporting guidelines for biological and biomedical investigations: the MIBBI project. Nat Biotechnol. 2008;26(8):889-96.

STROBE-The STRengthening the reporting of Observational Studies in Epidemiology

von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP; STROBE Initiative. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. PLoS Med. 2007 Oct 16;4(10):e296.

Data reporting/sharing

McQuilton P, Gonzalez-Beltran A, Rocca-Serra P, Thurston M, Lister A, Maguire E, Sansone SA. BioSharing: curated and crowd-sourced metadata standards, databases and data policies in the life sciences. Database (Oxford). 2016 May 17;2016. pii: baw075.

Perez-Arriaga MO, Wilson S, Williams KP, Schoeniger J, Waymire RL, Powell AJ. Omics Metadata Management Software (OMMS). Bioinformation. 2015;11(4):165–172.

Schumacher A, Rujan T, Hoefkens J. A collaborative approach to develop a multi-omics data analytics platform for translational research. Appl Transl Genom. 2014;3(4):105–108.

Wolstencroft K, Owen S, Krebs O, Nguyen Q, Stanford NJ, Golebiewski M, Weidemann A, Bittkowski M, An L, Shockley D, Snoep JL, Mueller W, Goble C. SEEK: a systems biology data and model management platform. BMC Syst Biol. 2015;9:33.

All five biomarker category studies should at least report:

- Study design and sampling methods (indicate if possible setting and recruitment periods)
- Study size (number of groups and individuals/group) indicating how was assessed
- Diagnosis criteria and severity grade, indicating how was assessed
- Demographic and clinical data of participants
- Variables studied, statistical methods
- Limitations and potential bias

BIOMARKER CATEGORY SPECIFIC CDE RECOMMENDATIONS

- Since all five biomarker categories selected for study by this Working Group require analysis of human derived samples (stools, body fluids or tissues), a selection of guidelines to record and report pre-analytical variables that could impact downstream applications as well as some quality control recommendations are shown:

Preanalytical variables: Biospecimen reporting for improved study quality (BRISQ) and Sample PREanalytical Code (SPREC) expected to facilitate and consolidate international multicenter biomarker identification research and biospecimen research in the clinical Biobank environment

Moore HM, Kelly AB, Jewell SD, McShane LM, Clark DP, Greenspan R, Hayes DF, Hainaut P, Kim P, Mansfield EA, Potapova O, Riegman P, Rubinstein Y, Seijo E, Somiari S, Watson P, Weier HU, Zhu C, Vaught J. ***Biospecimen reporting for improved study quality (BRISQ)****. Cancer Cytopathol. 2011;119(2):92–101.*

Ellervik C, Vaught J. Preanalytical variables affecting the integrity of human biospecimens in biobanking. Clin Chem. 2015;61(7):914–934.

Betsou F, Lehmann S, Ashton G, Barnes M, Benson EE, Coppola D, DeSouza Y, Eliason J, Glazer B, Guadagni F, Harding K, Horsfall DJ, Kleeberger C, Nanni U, Prasad A, Shea K, Skubitz A, Somiari S, Gunter E; International Society for Biological and Environmental Repositories (ISBER) Working Group on Biospecimen Science. Standard preanalytical coding for biospecimens: defining the sample PREanalytical code. Cancer Epidemiol Biomarkers Prev. 2010;19(4):1004–1011.

Betsou F, Barnes R, Burke T, Coppola D, Desouza Y, Eliason J, Glazer B, Horsfall D, Kleeberger C, Lehmann S, Prasad A, Skubitz A, Somiari S, Gunter E; [International Society for Biological and Environmental Repositories (ISBER) Working Group on Biospecimen Science]. Human biospecimen research: experimental protocol and quality control tools. Cancer Epidemiol Biomarkers Prev. 2009;18(4):1017–1025.

Sample quality control (QC):

ISBER (International Society for Biological and Environmental Repositories; http://www.isber.org):
Table 9. QC tools for samples used in proteomics, metabolomics, transcriptomics, or targeted analytical applications
Table 10. QC tools for molecular and cellular derivatives

Feng H, Zhang X, Zhang C. mRIN for direct assessment of genome-wide and gene-specific mRNA integrity from large-scale RNA-sequencing data. Nat Commun. 2015;6:7816.

Study design, study size assessment and statistical analysis:

Wallstrom G, Anderson KS, LaBaer J. Biomarker discovery for heterogeneous diseases. Cancer Epidemiol Biomarkers Prev. 2013;22(5):747–755.

Gosho M, Nagashima K, Sato Y. Study designs and statistical analyses for biomarker research. Sensors (Basel). 2012;12(7):8966–8986.

1. Microbiome/Microorganisms

International Human Microbiome Standards (IHMS) project: *http://www.microbiome-standards.org/#SOPS*

The Microbiome Quality Control project: *http://www.mbqc.org/*
NIH Human Microbiome Project: *https://www.hmpdacc.org/hmp/publications.php*
NIH Human Microbiome Project Tools and Technology: *https://www.hmpdacc.org/resources/tools_protocols.php*

Conceição-Neto N, Zeller M, Lefrère H, De Bruyn P, Beller L, Deboutte W, Yinda CK, Lavigne R, Maes P, Van Ranst M, Heylen E, Matthijnssens J. Modular approach to customise sample preparation procedures for viral metagenomics: a reproducible protocol for virome analysis. Sci Rep. 2015;5:16532.

Plaza Onate F, Batto JM, Juste C, Fadlallah J, Fougeroux C, Gouas D, Pons N, Kennedy S, Levenez F, Dore J, Ehrlich SD, Gorochov G, Larsen M. Quality control of microbiota metagenomics by k-mer analysis. BMC Genomics. 2015;16:183.

Vandeputte D, Tito RY, Vanleeuwen R, Falony G, Raes J. Practical considerations for large-scale gut microbiome studies. FEMS Microbiol Rev. 2017;41(Supp_1):S154–S167.

Microbiome/Microorganisms should at least report:

- Sample collection, transport and storage
- Nucleic acid extraction methods
- Any amplification (details)
- Next-Generation Sequencing (NGS) protocol [Whole Genome Shotgun Sequencing(WGSS), rRNA]
- NGS platforms
- Sequence analysis & pipelines

2. Proteome/Proteins

MIAPE (Minimal Information about a Proteomics Experiment; *http://psidev.info/miape*)

Martínez-Bartolomé S, Binz PA, Albar JP. The Minimal Information about a Proteomics Experiment (MIAPE) from the Proteomics Standards Initiative. Methods Mol Biol. 2014;1072:765–780.

Proteome/Proteins studies should at least report:

- Biological material that was extracted, how it was collected and stored
- Extraction procedure—reagents, pH, detergent concentration, resultant protein concentration and how protein concentration was determined
- How digestion and cleanup was carried out
- What quality control is performed on the mass spectroscopy (MS) system
- Brand and model of mass spec system
- Separation column used
- How long a gradient was used
- Pooling strategy of samples

3. Metabolome/Metabolism

The Metabolomics Standards Initiative (MSI): *http://www.metabolomics-msi.org/*
Core Information for Metabolomics Reporting (CIMR): *http://msi-workgroups.sourceforge.net*

Data reporting/sharing:

Haug K, Salek RM, Steinbeck C. Global open data management in metabolomics. Curr Opin Chem Biol. 2017;36:58–63.

Ara T, Enomoto M, Arita M, Ikeda C, Kera K, Yamada M, Nishioka T, Ikeda T, Nihei Y, Shibata D, Kanaya S, Sakurai N. Metabolonote: a wiki-based database for managing hierarchical metadata of metabolome analyses. Front Bioeng Biotechnol.2015;3:38.

Metabolome studies should at least report:

- Biological material that was extracted, how it was collected, stored and prepared for analysis
- Extraction procedure – solvent, instrumentation (e.g. magnetic/mechanical stirrer, sonication, temperature, duration)
- Whether and what type of cleanup was carried out, e.g., filtration, centrifugation, concentration or complete evaporation and re-uptake in a different solvent
- How samples are stored – temperature, duration
- What quality control is performed on the MS system, e.g., standards used

- Brand and model of mass spec system, ionization mode, brand and model of high performance liquid chromatography (HPLC) system
- Separation column used
- Solvent gradient parameters

Data evaluation, quantitation and validation:

- Targeted or non-targeted analysis
- Relative quantitation or absolute quantitation (using stable isotope labeled internal standards)
- MS/MS validation using multiple fragment ions for quantitation plus qualifier fragment ions when needed

Together with MS I think it is fair to include nuclear magnetic resonance (NMR) as a complementary technique, including all parameters for this method.
As complement to liquid chromatography (LC) we should mention gas chromatography (GC), capillary electrophoresis (CE), capillary electrochromatography (CEC) and super critical fluid chromatography (SFC) as other separation techniques commonly used in metabolomics.
Maybe also something about the statistics used for data evaluation together with bioinformatics tools applied.

4. Genome/Epigenome

*Minimum Information about a Genotyping Experiment (**MIGEN**)*

Huang J, Mirel D, Pugh E, Xing C, Robinson PN, Pertsemlidis A, Ding L, Kozlitina J, Maher J, Rios J, Story M, Marthandan N, Scheuermann RH. Minimum Information about a Genotyping Experiment (MIGEN). Stand Genomic Sci. 2011;5(2):224–229.

MINSEQE (Minimum Information about a high-throughput SEQuencing Experiment): *http://www.fged.org/projects/minseqe/*

STrengthening the REporting of Genetic Association Studies (STREGA)

Little J, Higgins JP, Ioannidis JP, Moher D, Gagnon F, von Elm E, Khoury MJ, Cohen B, Davey-Smith G, Grimshaw J, Scheet P, Gwinn M, Williamson RE, Zou GY, Hutchings K, Johnson CY, Tait V, Wiens M, Golding J, van Duijn C, McLaughlin J, Paterson A, Wells G, Fortier I,

Freedman M, Zecevic M, King R, Infante-Rivard C, Stewart A, Birkett N; STrengthening the REporting of Genetic Association Studies. STrengthening the REporting of Genetic Association Studies (STREGA): an extension of the STROBE statement. PLoS Med. 2009 Feb 3;6(2):e22.
Table 1 STREGA Reporting Recommendations, Extended from STROBE Statement

Genome/Epigenome studies should at least report:

- Source of DNA (cells, tissue, etc. and how was prepared)
- How DNA was prepared and stored
- DNA quality parameters
- Method and platform (type and vendor) used to obtain expression levels
- How bioinformatic analysis was performed

5. Gene expression/Transcriptome

The **MIQE guidelines**: minimum information for publication of quantitative real-time PCR experiments
Bustin SA, Benes V, Garson JA, Hellemans J, Huggett J, Kubista M, Mueller R, Nolan T, Pfaffl MW, Shipley GL, Vandesompele J, Wittwer CT. The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. Clin Chem. 2009;55(4):611–622.

Huggett JF, Foy CA, Benes V, Emslie K, Garson JA, Haynes R, Hellemans J, Kubista M, Mueller RD, Nolan T, Pfaffl MW, Shipley GL, Vandesompele J, Wittwer CT, Bustin SA. The digital MIQE guidelines: Minimum Information for Publication of Quantitative Digital PCR Experiments. Clin Chem. 2013;59(6):892–902.

Minimum Information About a Microarray Experiment (**MIAME**)
*Brazma A. Minimum Information About a Microarray Experiment (**MIAME**)-successes, failures, challenges. ScientificWorldJournal. 2009;9:420–423.*

Gene expression/Transcriptome studies should at least report:

- Source of RNA (cells, tissue, etc. and how was prepared)
- How RNA was prepared and stored
- RNA quality parameters
- Method and platform (type and vendor) used to obtain expression levels
- How bioinformatic analysis was performed

Technology for gene expression analysis is developing rapidly. Cost considerations are significant and some investigators will be limited to certain platforms. Most popular among currents are:

Biased (targeted): Quantitative Polymerase Chain Reaction following Retrotranscription (qRT-PCR), microarrays, differential display, etc.
Unbiased (non-targeted): Next Generation sequencing (NGS) based strategies (various vendors)

Data quality control and Data reporting/sharing:

Blondal T, Jensby Nielsen S, Baker A, Andreasen D, Mouritzen P, Wrang Teilum M, Dahlsveen IK. Assessing sample and miRNA profile quality in serum and plasma or other biofluids. Methods. 2013;59(1):S1–S6.

DeLuca DS, Levin JZ, Sivachenko A, Fennell T, Nazaire MD, Williams C, Reich M, Winckler W, Getz G. RNA-SeQC: RNA-seq metrics for quality control and process optimization. Bioinformatics. 2012;28(11):1530–1532.

Navarange M, Game L, Fowler D, Wadekar V, Banks H, Cooley N, Rahman F, Hinshelwood J, Broderick P, Causton HC. ***MiMiR****: a comprehensive solution for storage, annotation and exchange of microarray data. BMC Bioinformatics. 2005;6:268.*